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Silver doped titanium dioxide nanoparticles as antimicrobial additives to dental polymers

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ABSTRACT

Objectives: The objectives of this i*n vitro* study were to produce a filled resin containing Ag-TiO_2 filler particles and to test its antibacterial properties.

Methods: Ag-TiO_2 particles were manufactured using the ball milling method and incorporated into an epoxy resin using a high speed centrifugal mixer. Using UV/Vis spectrophotometry investigations were performed to assess how the photocatalytic properties of the Ag-TiO_2 particles are affected when encased in resin. Adopting the bacteria colony counting technique, the antibacterial properties of Ag-TiO_2 particles and Ag-TiO_2 containing resins were assessed using *S. mutans* under varying lighting conditions.

Results: Ag doping of TiO_2 results in a band gap shift towards the visible spectrum enabling Ag-TiO_2 to exhibit photocatalytic properties when exposed to visible light. Small quantities of Ag-TiO_2 were able to produce a bactericidal effect when in contact with *S. mutans* under visible light conditions. When incorporated into the bulk of an epoxy resin, the photocatalytic properties of the Ag-TiO_2 particles were significantly reduced. However, a potent bactericidal effect was still achieved against *S. mutans*.

Significance: Ag-TiO_2 filled resin shows promising antimicrobial properties, which could potentially be used clinically.

1. Introduction

A truly antibacterial resin could find a number of clinical dental applications in both restorative dentistry and orthodontics. In restorative dentistry it could be used as a filling or denture base material, whilst in orthodontics it could be used as a bracket or bracket bonding material. Demineralisation of enamel is still one of the main complications of orthodontic treatment, particularly with fixed appliances [1]. The first sign of demineralisation may be the development of white spot lesions (WSL) on the enamel surface around the bracket margins, which if left unchecked can progress to cavitation. This whole process occurs more rapidly in orthodontic patients when compared to non-orthodontic patients [2,3]. Preventing enamel demineralisation and the formation of WSL is an important consideration for clinicians, as the lesions are unaesthetic and potentially irreversible. One cross sectional study showed that up to 50% of individuals undergoing fixed appliance therapy had non-developmental WSL compared with just 25% of controls [4]. Although the progression from WSL to cavitation is low (cavitation occurs in only 2% of WSL), the high incidence of WSLs is a significant factor where orthodontic treatment is being performed to improve aesthetics [5].

A Cochrane review assessing the effectiveness of methods used in the prevention of WSL formation concluded there was only evidence to support the use of daily 0.05% sodium fluoride mouthrinses in order to reduce their prevalence and severity [6]. Unfortunately it is often the case that patients at the highest risk of developing WSL are those least likely to comply with oral hygiene and mouth rinsing regimes. Therefore less patient dependent modes of delivery are required. Although the same Cochrane review concluded that the use of glass-ionomer cement for bracket bonding reduces the prevalence and severity of WSL,

both glass-ionomer and resin modified glass-ionomer cements (RMGIC) have not gained widespread acceptance as orthodontic bonding agents, due to the reportedly lower shear bond strength and a lack of familiarity when compared to resin bonding systems and the associated acid etch technique [7].

A number of new technologies, principally fillers and coatings, have recently become available with potential antimicrobial properties. Although such coatings have the potential to be effective, they are also likely to be abraded and lost over time, or may corrode within the oral environment. For example if orthodontic brackets were to be coated with an antibacterial material it is unlikely it would be maintained on the bracket surface for the duration of a course of treatment [8].

Titanium dioxide (TiO_2), particularly in its nanoparticle form, has generated a great deal of interest over recent years, as it has numerous potential applications. A number of studies have demonstrated it to be an effective light activated photocatalyst, with strong bactericidal activity. However, its main disadvantage is its wide band-gap, meaning it is only really bactericidal when it absorbs UV light. With UV light being only a very small fraction of the solar spectrum (<5%), it means its activity is severely reduced under dark or visible light conditions, as would be found within the oral cavity [9]. In addition, traditional TiO_2 photocatalysis is effective only upon irradiation with UV-light at levels that could cause damage to human cells. As a result, researchers have conducted extensive studies on doping, sensitisation and covering the surface of the TiO_2 with dyes, in order to extend light absorption to the visible range. Doping $TiO_2$with transition metal ions and/or anions is commonly used to this effect. This method creates intra-band gap states close to the conduction or valence band edges that induce visible-light absorption at the sub-band gap

energies [10]. Ag^+ modification of TiO_2 induces a decrease in the band gap energy, allowing visible light to activate the material's photocatalytic activity. This opens the possibility that such technology could be adopted within dental biomaterials and help prevent bacterial colonisation of intraoral appliances. Examples might include polymeric orthodontic brackets, bonding resins, dentures and intracoronal polymeric restorations. If the active particles were retained within the bulk of a polymer as well as at the surface, then any wear would simply expose more particles and thereby continue to confer an antimicrobial effect.

In this study the aims were to examine the effects of incorporating doped titanium dioxide nanoparticles into a polymer. The specific objectives were to investigate:

1. Free radical release at different silver doping concentrations of TiO_2 nanopowder.

2. The effect of varying the light conditions has on free radical release.

3. Free radical release when Ag-TiO_2 particles are incorporated into a bulk polymer.

4. The effect of silver doped TiO_2 on bacterial growth, both as a powder and when incorporated within a bulk resin

2. Materials and methods

2.1 Production of Ag-TiO_2 powders: Silver doped photocatalytic TiO_2 powders were prepared by ball milling from commercial TiO_2 (P25) powders. Four different suspensions of Ag-TiO_2, were produced (Table 1). After drying, the powders were calcined at 400°C for 90 minutes in a high temperature oven (Heratherm Oven, Thermo Scientific, UK) in order to decompose the silver salt and permit diffusion of the silver ions (Ag^+). Following calcination,

the course powder produced was further ground using a centrifugal laboratory mixing and grinding machine (DAC150 Speedmixer, Hauschild Engineering, High Wycombe, UK).

To assess how the silver loading affected the band gap energy of the TiO_2, the powder samples were placed in a UV/Vis Spectrophotometer (Lambda 35 UV/Vis Spectrophotometer, Perkin Elmer, Massachussets, USA) and the readings plotted to show absorption at wavelengths ranging from 200 to 500nm. Optical spectra were analysed using the Tauc model as reported by Impellizeri *et al*. (2015)[11]. Using this data the band gap shift for the TiO_2 and each of the four Ag-TiO_2 powders (2, 4, 6, and 8%) was determined.

2.2 Testing Ag-TiO_2 powder for free radical release under different lighting conditions: The Ag-TiO_2 powders were next tested for free radical release under visible light conditions. 0.05g of samples 1 to 4 (Table 1) of Ag-TiO_2 powder and TiO_2 powder were added to beakers containing 10ml of Methylene Blue dye used as a photobleaching indicator of photcatalysis [12]. These aqueous solutions were left stirring for 4 hours under visible light conditions of 2000 lux with the light intensity measured using a digital light lux meter (Model DT-1300, CEM, Shenzen, China). Three samples of 50µl of each solution were collected every 30 minutes for 4 hours and dispensed into a microplate and stored under dark conditions. The microplate was then positioned in the spectrophotometer to measure the absorbance of the solutions in each of the wells. The same procedure was carried out under dark and UV light (355nm) conditions.

2.3 Incorporation of silver doped titanium dioxide into an unfilled resin and testing for free radical release: The 6% Ag doped TiO_2 powder was shown to have the most effective photocatalytic properties under visible light conditions and was subsequently incorporated into the polymeric samples. These samples were made using 3.5g of uncured epoxy resin, 1g

of curing agent and 3.6g of 6% Ag doped TiO_2 powder to produce a 44% by weight Ag-TiO_2 resin sample. The uncured epoxy and doped powder were placed into the centrifugal laboratory mixing and grinding machine and spun at 3000rpm for 5 minutes to ensure adequate dispersion of the powder within the resin. The curing agent was then added and the specimen mixed at 2500rpm for a further 30 seconds. The final mixed resin was then poured into a plastic mould measuring 3.5cm in diameter and 0.5cm thickness. At this degree of filler loading it was possible to incorporate the powder in the resin, mix it and pour it into the moulds.

To test for free radical release the 6% Ag loaded Ag-TiO_2 resin disk was then placed in a 100ml glass beaker containing 10ml of Methylene Blue dye and stored under visible light conditions of 2000 lux. Three samples of 50µl of each solution were collected every 30 minutes for 4 hours using an Eppendorf micropipette and placed into a microplate well, which was stored under dark conditions. At the end of collection, the microplate was positioned in the spectrophotometer, which had previously been calibrated to a wavelength of 655nm (visible light setting), in order to measure the absorbance of each of the Methylene Blue solutions. An average of the 3 readings was calculated for each solution and plotted on a line graph showing the reduction of Methylene Blue dye with time in the presence of Ag-TiO_2 resin.

To determine the effect of an increased surface area of available Ag-TiO_2 particles in the resin on free radical release, a further 6% Ag doped Ag-TiO_2 resin disk was sectioned into 8 smaller segments, thereby increasing the surface area from 25cm^2 to 39cm^2.

2.4 Antibacterial testing: In order to test the likely effectiveness of the Ag+ doped TiO_2 powders against cariogenic bacteria, *S. mutans* strain NG8 was chosen. 0.05, 0.1 and 0.2g of

6% Ag-TiO_2 powder was placed into three separate wells, with a fourth well left empty to act as a negative control. To each of the four wells, 0.9ml of sterile PBS (phosphate-buffered saline: 10 mM KH_2PO_4-K_2HPO_4 at pH 7.2 containing 0.15 M NaCl) was added along with 0.1 ml of *S. mutans* cell suspension (OD600 1.0). The plate was then positioned on a rocking platform (Stuart See-saw rocker, Bibby Scientific Ltd, Staffs, UK) under an LED light for 4 hours and at a distance that provided an intensity of 1500 lux. A LED light was used to ensure no heat was given off. A separate well containing 0.05 g of 6% Ag-TiO2 powder in 0.9 ml of PBS and 0.1 ml *S. mutans* culture was also placed under dark conditions for the same time period in order to act as a control.

At 4 hours, the plate was removed from the light source and 0.1 ml samples were removed from the wells. Each was serially 10-fold diluted in PBS and portions (20 µl) were spotted onto BHY agar (37 g/L Brain Heart Infusion, 5 g/L yeast extract, 15 g/L agar) plates. These were incubated for 16hrs at 37^oC in a candle jar and colonies were counted in order to estimate the viable numbers of *S. mutans* cells expressed as colony forming units (CFU). The experiments were carried out in duplicate

The same experimental procedure was carried out using an Ag-TiO_2 resin sample, fabricated as describes in section 2.3. A 6% Ag-TiO_2, filled resin disk, 3.5cm in diameter, was cut into 5mm^2 sections. One 5mm sample was then placed in a well containing 0.9 ml of sterile PBS, together with 0.1 ml of *S. mutans* cell suspension, positioned on a rocking platform and exposed to LED light of 1500 lux intensity for 4 hours. A second 5mm section was placed in a well containing bacterial suspension in PBS on a rocking platform and covered with a foil lid, preventing light access and acting as a control.

3. Results

3.1 UV/Vis spectrometry of TiO_2 and Ag-TiO_2 powders: The UV/Vis spectra were analysed using a Tauc model. The results were plotted and are illustrated in Figure 1. It can been seen that the band gap of Ag doped TiO2 shifted towards the lower visible light region, from 3.1 eV (undoped TiO_2) to 2.6 eV (6% and 8% Ag doped TiO_2).

Methylene Blue (MB) was used as an indicator of free radical release and therefore photoreduction. As expected the control solutions of MB, and MB with TiO_2 showed no reduction as TiO_2 particles only exhibit photocatalytic properties under UV light conditions. Light of visible wavelength does not have sufficient energy to overcome the band gap and induce a photocatalytic reaction. Unlike the pure TiO_2 powder, which dissolved readily in the MB, the remaining Ag-TiO_2 powders did not disperse fully, despite extensive grinding and ball milling in preparation. Instead some of the powder would collect at the base of the beaker. Photoreduction of the MB over the 4 hour period was seen with all of the Ag-TiO_2 powders, 2% through to 8% (Figure 2). The greatest reduction of the MB dye occurred between 60 and 120 minutes in the majority of the solutions. Overall the 6% Ag-TiO_2 powder achieved the most effective reduction of MB and over the shortest time period.

3.2 A comparison of 6% Ag-TiO_2 particles reduction of Methylene Blue under UV, visible and dark light conditions.

The data for the reduction of MB in the presence of 6% Ag-TiO_2 from each experiment can be seen in Figure 3. It shows that if no light comes into contact with the particles then reduction of the MB will not occur.

When visible light and UV light were applied to the 6% Ag doped Ag-TiO_2 particles, a similar reduction of MB were seen over the 4 hour experimental period, with both solutions

reducing in absorbance from 1.6 to 0.3. However, reduction was achieved at a faster rate under UV light application, seen as a steeper initial curve up to 150 minutes. This curve then dramatically levelled off and no further reduction was seen for the remaining 90 minutes. The 6% Ag doped Ag-TiO_2 particles with visible light application showed a slow rate of reduction over the initial 150 minutes. However, the reduction continued over the final 90 minutes, reaching the same absorbance of 0.3 as the solution with UV light application.

3.3 Testing free radical release of the silver doped titanium dioxide filled resin

The effect of 6% Ag doped Ag-TiO_2 filled resin on the reduction of MB under visible light conditions is illustrated in Figure 4. The MB control solution showed no reduction in absorbance over the 4 hour experimental period as expected and neither did the polished 25cm^2 filled resin disc. When the surface area of the filled resin disc was increased to 39cm^2 by sectioning, a small reduction in the absorbance of the MB solution occurred from 0.6 to 0.5 over the 4 hour period of visible light application. This indicates that the increase in surface area has created a higher number of Ag-TiO_2 particles exposed at the surface of resin, allowing them to retain some of their photocatalytic properties. However, the effects are much less than was observed with the powder on its own.

3.4 Antibacterial testing of Ag-TiO_2 powders under visible light and dark conditions

The potential antibacterial effect of 6% Ag doped Ag-TiO_2 was tested using powder alone and powder embedded within a resin. Figure 5 shows the effect of the powder on *S. mutans* viability, expressed as CFU. It can be seen that the control solution, containing no Ag-TiO_2 powder, when exposed to visible light of 1500 lux from an LED lamp showed significant growth of *S. mutans.* Even in the most dilute solutions in the 10^{-5} quadrant, bacterial colonies were still clearly visible. The solutions containing 0.05g, 0.1g and 0.2g of 6% Ag

doped Ag-TiO_2 particles all showed no bacterial growth occurred even at the greatest bacterial concentrations.

Complete bacterial killing was seen in all suspensions containing Ag-TiO_2 particles that were exposed to visible light conditions, regardless of the volume of Ag-TiO_2 particles present (0 bacterial per ml**)**, as seen in Figures 5b and c. The control suspension (Figure 5a) agar plate clearly showed bacterial colonies at the highest dilution, calculated as 4.6 x 108 bacteria /ml. Bacterial growth was also seen from the suspension containing 0.05g of 6% Ag doped Ag-TiO_2 particles when it was kept under dark conditions (Figure 5d). However, colonies were not present above the 10^3 dilution, with a maximum CFU of 6 x 10^5 bacterial/ml. This indicates a nearly 3-log reduction in CFU compared to control, so bacterial killing was occurring in the suspensions containing Ag-TiO_2 particles alone, in the absence of the photocatalytic reaction and free radical action.

3.5 Antibacterial testing of 6% Ag doped Ag-TiO_2 filed resin under visible light and dark conditions

In these experiments, 6% Ag doped Ag-TiO_2 filled resin samples were polished, cut into 5 mm sections and placed in contact with *S. mutans* as before Figure 6 shows *S. mutans* colony growth on agar plates for suspensions that had been in contact with the 6% Ag doped Ag-TiO_2 resin, one suspension kept in dark conditions and the other exposed to visible light.

The suspension that was kept in dark conditions showed clear *S. mutans* CFU up to the 10^4 dilution, with CFU calculated as 1.6 x 107 bacteria/ml (Figure 6a). By contrast, total bacterial killing occurred in the suspension containing a 5 mm section of Ag-TiO_2 resin disk exposed to visible light conditions of 1500 lux for 4 hours (Figure 6b).

4. Discussion

In order for TiO_2 to be an effective bactericide in the presence of visible light within any biomaterial it is necessary to shift the band gap energy towards the visible spectrum of light. In the current experiment the use of Ag doping led to a measurable band gap shift towards the visible spectrum, beginning at around 390 nm. This can be attributed to the local structure of TiO_2 being altered by Ag doping [13]. This shift was dose related with an increasing shift from 2% up to 6%. However, when the Ag loading was increased to 8% this trend did not continue. The band gap shift was still towards the visible spectrum, but not to the same degree as the 6% doped Ag-TiO_2. This finding was in contrast to other studies, which found the band gap shift continued towards the visible spectrum as the Ag loading of TiO_2 continued to increase [14, 15]. However, these studies manufactured Ag-TiO_2 films using a hybrid sol–gel method, not ball milling, and so the findings cannot be directly compared. Nevertheless**,** our findings highlight the importance of adjusting the Ag and TiO2 composition to the correct ratio in order to acquire the optimum photocatalytic properties.

4.1 Free radical release at different Ag loadings

Methylene Blue, a cationic dye, is widely used as a redox indicator in analytical chemistry and the photocatalytic efficiency of other Ag-TiO_2 powders has been successfully tested using this technique [12,16]. The results showed 6%wt Ag loaded TiO_2 over 4 hours appeared to produce the greatest effect, with the final solution being almost completely clear following exposure. This was confirmed using spectrophotometry. Other studies have found that around 2% wt Ag loading of TiO_2 produces Ag-TiO_2 compounds with the most efficient photocatalytic properties under visible light conditions [16, 17]. This is lower than the 6%wt doping found in our study and a possible reason for this is the use of different

doping methods, notably the sol-gel and laser-liquid interaction methods rather than the ball milling used in our experiment.

Undoped TiO_2 nanoparticles have been successfully incorporated into dental composites and have been shown to improve mechanical properties by increasing the strength and reducing the porosity of the material [18]. However, no clinical trials have been carried out to assess whether or not these nanofiller particles provide the material with any antimicrobial properties. Certainly no photocatalytic properties would be expected as UV light application would be necessary to induce redox reactions at the surface of the particles, which would be damaging to human cells. To date Ag-TiO_2 nanoparticles have not yet been incorporated into dental composites, but they do have the potential to provide long term antimicrobial activity to such materials. The potential advantage of a Ag-TiO_2 nanofilled composite is that it not only provides bactericidal activity via photocatalysis when exposed to visible light in the anterior regions of the mouth, but it is also a bactericidal agent in its own right [19]. By applying visible light in the form a dental curing light (450-490nm wavelength) for short periods of time at each patient visit, it might be possible to boost the material's photocatalytic antibacterial activity even further. This could help to prevent bacterial colonisation of orthodontic brackets and resin based adhesives and restorative materials and help to reduce the occurrence of caries, particularly if it is retained as a bulk material throughout a course of treatment. It also overcomes patient compliance issues. What still needs to be assessed is how the photocatalytic properties are affected by incorporating the Ag-TiO_2 particles into the bulk of a resin and how the aesthetic properties of the resin are affected.

4.2 Free radical release of the silver doped titanium dioxide filled resin

The photocatalytic properties of Ag-TiO_2 particles appear to be directly related to the surface area of the material exposed to light. The larger the surface area available, the greater the photocatalytic properties [20]. The initial experiment using Ag-TiO_2 particles embedded in epoxy resin showed that the photocatalytic properties of the powder had been completely hindered. However, when the surface area was increased from 25% to 39%, some reduction of the MB was evident. This was a promising finding as it showed that some of the Ag-TiO_2 photocatalytic properties could be retained following incorporation into a bulk resin.

When considering the clinical applications of a Ag-TiO_2 containing resin these include orthodontic and restorative treatments. In the case of orthodontics, only a very small surface area of bonding resin is exposed around the bracket base, which may lower the Ag-TiO_2 content to levels unable to achieve the desired photocatalytic effect. However, as in restorative applications, regular tooth brushing may continually refresh the resin surface, exposing the embedded Ag-TiO_2 particles and potentially helping to promote the photocatalytic effects for the duration of a course of treatment. Indeed the photocatalytic action is only required at the periphery of orthodontic brackets, where it is close to the exposed enamel surface and where any potential WSL is likely to occur.

4.3 Antibacterial testing of Ag-TiO_2 powders under visible light and dark conditions

Fixed orthodontic appliances not only make conventional oral hygiene procedures more difficult, they also increase the number of plaque retentive sites. This can lead to a rapid shift in the bacterial composition of dental plaque, most notably in the number of acidogenic bacteria, such as *S. Mutans* [2, 21] and this shift may persist throughout and

beyond a course of treatment [22]. The first sign of demineralisation may be the development of WSL on the enamel surface around the bracket margins, which if left unchecked can progress to cavitation. This whole process occurs more rapidly in orthodontic patients when compared to the development of similar lesions in non-orthodontic patients [2, 3].

Although the current study had shown that Ag-TiO_2 powder, and to a lesser extent when the powder is embedded in resin, can be an effective visible light driven photocatalyst, it was felt important to assess the bactericidal effects against pathogenic bacteria, in this case *S. mutans* NG8. Complete bacterial killing was seen in all suspensions containing Ag-TiO_2 powder, even at low concentrations following exposure to visible light. Under dark conditions the number of CFU were still reduced compared with untreated controls. This was an interesting finding as it indicated a proportion of the bactericidal effect could not be attributed purely to the photocatalytic properties of the Ag-TiO_2 particles. This may have been due to free silver not fully integrated within the TiO_2 crystal structure. If Ag^+ ions were subsequently released they could cause protein denaturation and inactivation within bacterial cell walls, leading to cell death [19, 23].

It was promising to find that the Ag-TiO_2 particles retained some, if not all of their bactericidal activity after being incorporated into the bulk of a resin. Figure 6 shows the bacterial growth when *S. mutans* came into contact with Ag-TiO_2 resin, under visible light or dark conditions. Again complete bacterial killing was seen in the suspension exposed to visible light, indicating the photocatalytic properties were retained after incorporation of the particles into the bulk resin. This might purely have been as a result of activation of Ag-TiO_2 particles at the resin surface, or as a result of light transmission through the resin. Any

porosity within the resin may have also permitted access to the Ag-TiO_2 particles or free Ag+ ions from unincorporated silver. Although many more bacteria survived the dark conditions in these experiments, there was still approximately ten-fold killing. These two potential mechanisms of bacterial killing would be advantageous within the clinical setting. If the particles were incorporated into a bonding resin, or a polymeric bracket or restorative material, then it can be assumed that the anterior regions of the mouth would be the only areas exposed to enough visible light to elicit a photocatalytic response. Anterior teeth, which are the most susceptible to WSL formation during orthodontic treatment [4] would benefit from both the photocatalytic activation and hence bactericidal effect of the Ag-TiO_2 and also the direct effect of any free silver and therefore Ag+ ions released. Teeth in the posterior regions of the mouth would be unlikely to receive enough visible light to produce a photocatalytic response from the Ag-TiO_2 particles, but would still benefit from the local ionised Ag. The photocatalytic properties could potentially be boosted in the posterior regions by the manual application of a visible light source. Ideally it would produce light at the low end of the visible spectrum, around 400-450nm, which would activate the full bactericidal effects of the Ag-TiO_2 particles. Further work would need to be carried out to assess how regularly and for what length of time this light would need to be applied in order to produce a consistent photocatalytic effect that would kill pathogenic oral bacteria.

How the physical properties of any resin might be affected by incorporation of Ag-TiO_2 particles has not been assessed in this study. Although studies have shown that the incorporation of antibacterial agents, such as chlorhexidine can dramatically reduce bond strength [26], other have shown that incorporation of TiO_2 nanoparticles also improves the microhardness and flexural strength of dental composites [27]. It may be that Ag-TiO_2

particles not only provide adhesive resins with inherent antimicrobial properties, but also might simultaneously improve mechanical properties.

5. Conclusions

This study has demonstrated that:

- Ag doping of TiO_2 results a band gap shift towards the visible spectrum that enables the Ag-TiO2 nanoparticles to exhibit photocatalytic properties when exposed to visible light.
- Following ball milling, 6%wt Ag doped TiO_2 demonstrated the most effective photocatalytic properties under visible light conditions.
- Even small quantities of Ag-TiO_2 nanoparticles (>2%wt) were able to produce a significant bactericidal effect when in contact with *S. mutans* under visible light.
- A portion of the Ag-TiO_2 bactericidal effects against *S. mutans* were retained under dark conditions.
- When incorporated into the bulk of an epoxy resin, the photocatalytic properties of the Ag-TiO_2 nanoparticles significantly reduced.
- The Ag-TiO_2 filled resin was still able to produce a complete bactericidal effect when in contact with *S. mutans* under visible light conditions.

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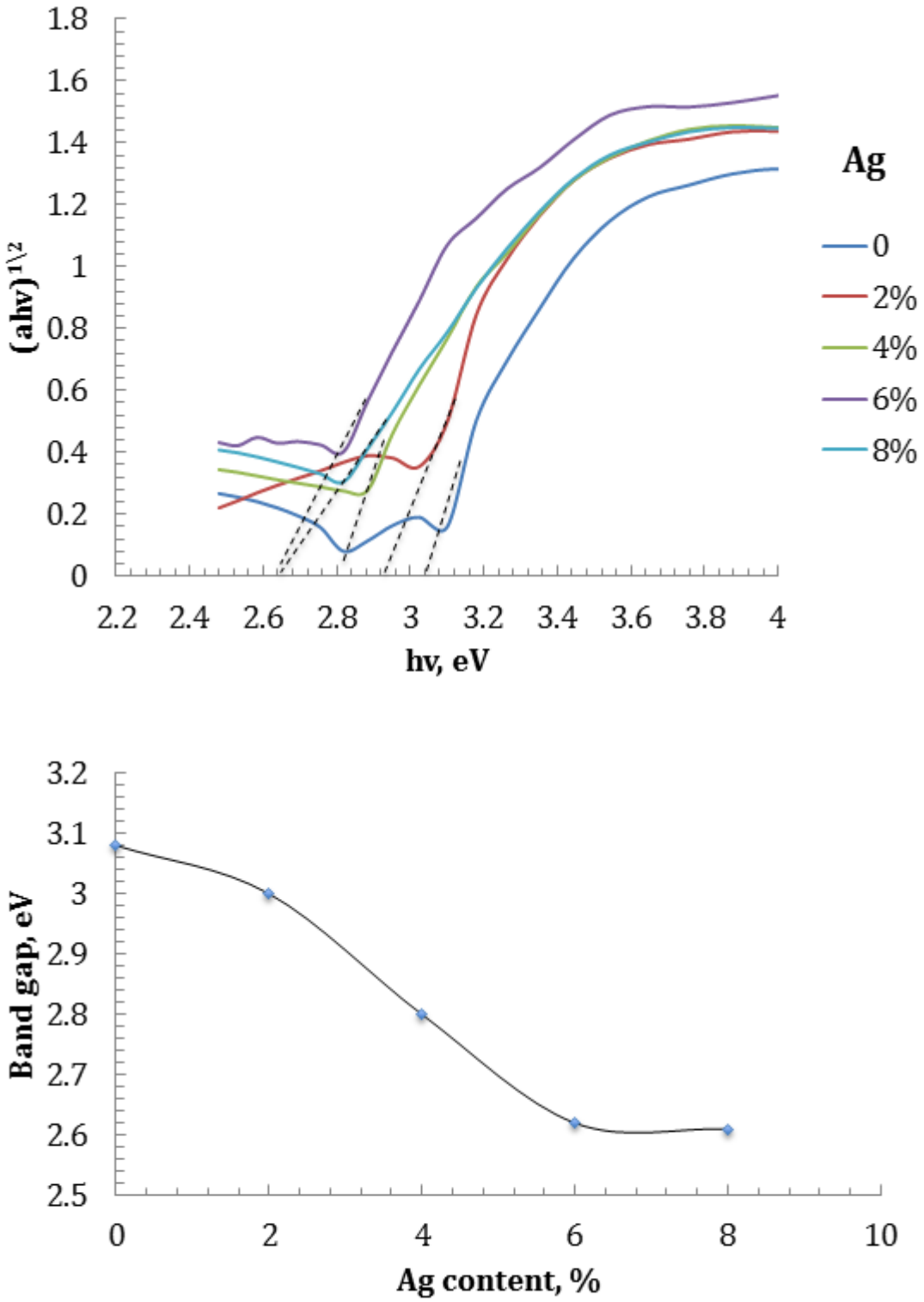

Figure 1. **a.** Tauc plots of undoped and Ag-doped TiO_2 powders; **b.** Band gap change with Ag doping level, showing the band gap shift of Ag-doped TiO_2 towards the visible light region.

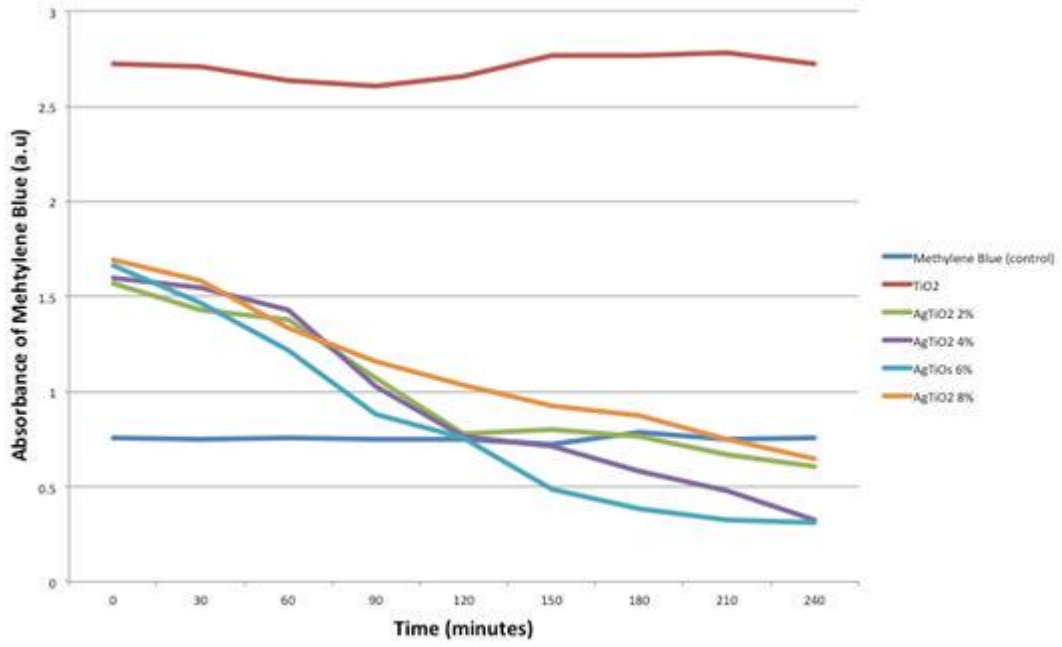

Figure 2. Absorbance of Methylene Blue (MB) with time in the presence of different concentrations of doped Ag-TiO_2 powders (2% to 8%) under visible light conditions.

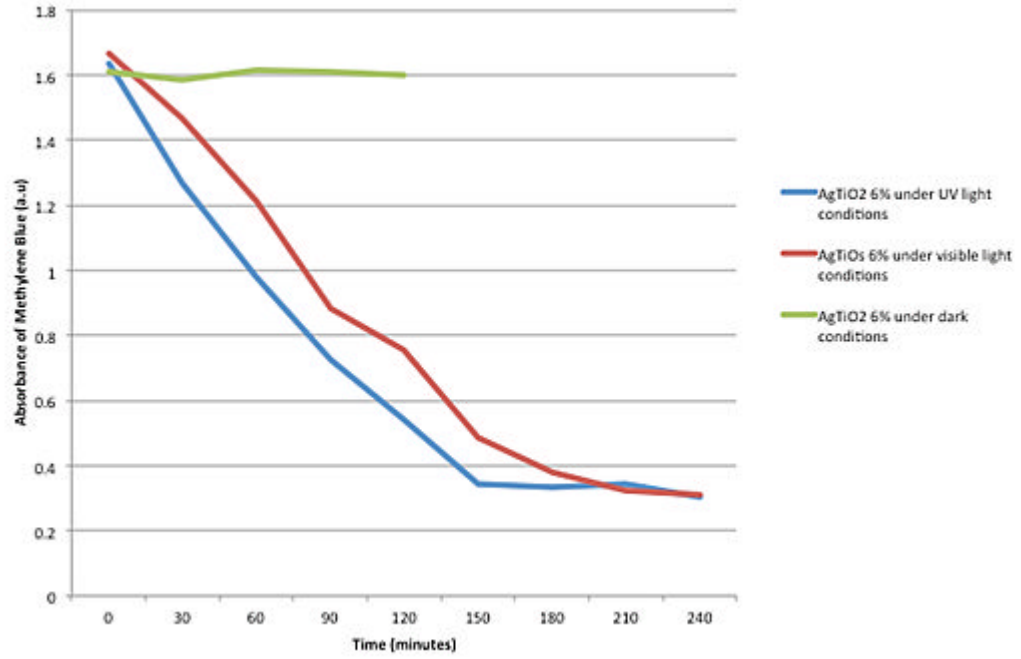

Figure 3. Absorbance of Methylene Blue in the presence of 6% Ag doped Ag-TiO_2 powder under UV, visible and dark light conditions

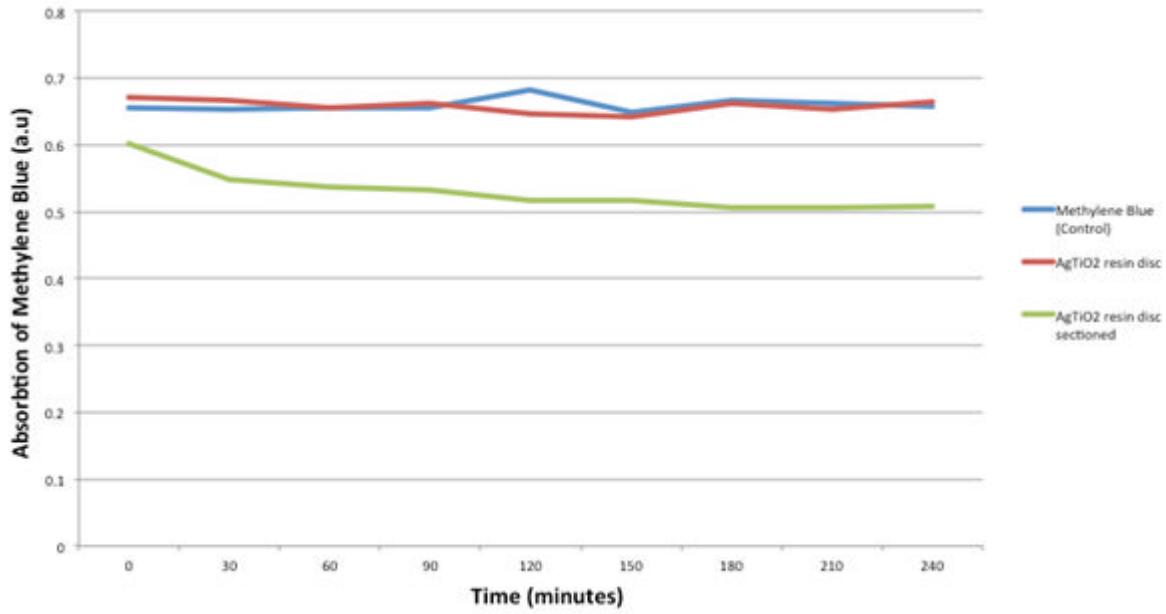

Figure 4. Graph showing absorbance of Methylene Blue with time under visible light conditions in the presence of 6% Ag doped Ag-TiO_2 resin discs.

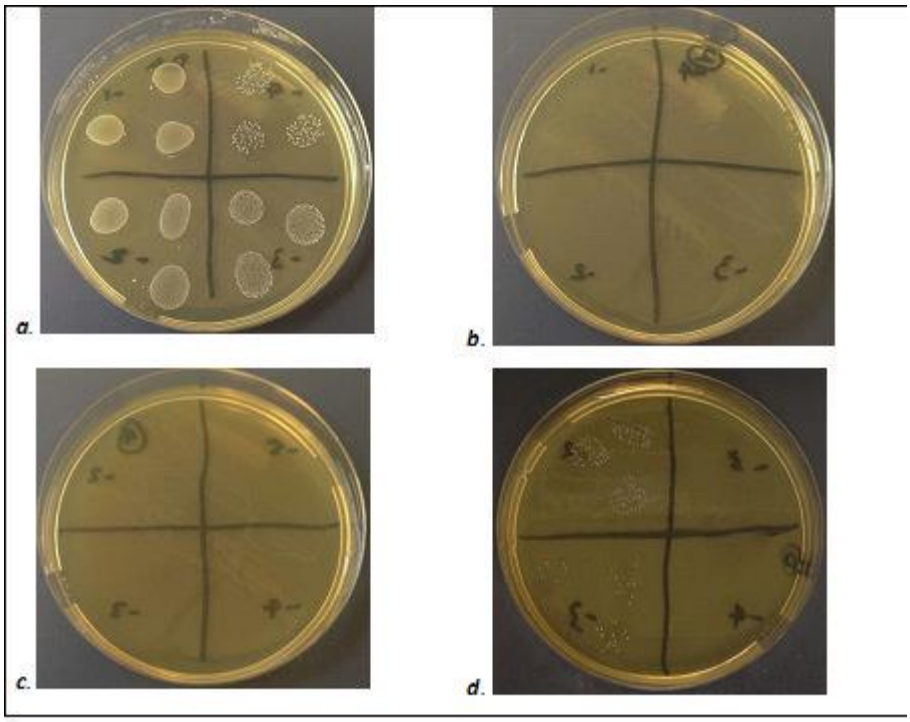

Figure 5. Agar plates displaying bacterial growth in varying dilutions of each solution containing *S. mutans* - **a**. control *S. mutans* exposed to visible light for 4 hours **b**. *S. mutans* in contact with 0.05g 6% Ag doped Ag-TiO_2 exposed to visible light for 4 hours **c**. *S. mutans* in contact with 0.1g 6% Ag doped Ag-TiO_2 exposed to visible light for 4 hours and **d**. *S. mutans* in contact with 0.05g 6% Ag doped Ag-TiO_2 kept in dark conditions

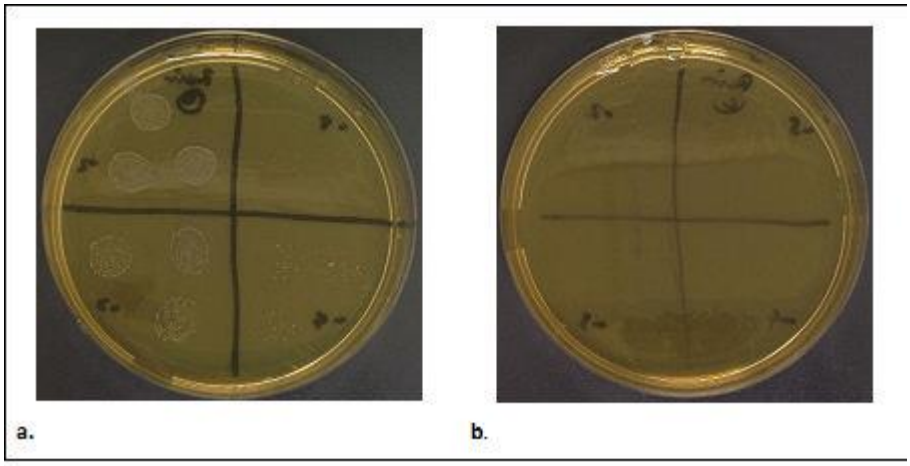

Figure 6. Agar plates displaying bacterial growth in varying dilutions of each suspension containing *S. mutans* and 5mm Ag-TiO_2 resin disk. **a.** *S. mutans* in contact with 5ml sectioned 6% Ag doped Ag-TiO_2 resin disk, kept under dark conditions **b**. *S. mutans* in contact with 5ml sectioned 6% Ag doped Ag-TiO_2 resin disk, exposed to visible light for 4 hours

Sample	$AgNO_3$	Na_2CO_3
1 = 2% Ag	2.3ml	2.5ml
2 = 4% Ag	4.6ml	5.0ml
3 = 6% Ag	6.9ml	7.5ml
4 = 8% Ag	9.2ml	10ml

Table 1. Four different volumes of $AgNO_3$ and Na_2CO_3 used for the construction of Ag-TiO_2 suspensions.